

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant: Paul Vermeij
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DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

I, Dr. Paul Vermeij, inventor of the invention claimed in the above identified Application, and Applicant therefor, hereby declare:

In the present application it is stated that "...*Lawsonia intracellularis* produces nine novel proteins, each of which is capable, separately or in combination with any of the other of these nine novel proteins, to produce immunity against *Lawsonia intracellularis*. The first of these nine novel proteins will be referred to as the 75 kD protein." (Specification, page 2, lines 15-20).

Particular reference to the 75 kD protein is disclosed in Example 1. "Conclusion: the 75 kD vaccine component could be successfully expressed in large quantities and is indeed clearly recognized by both orally challenged pig anti-*L. intracellularis* serum and by chicken anti-*L. intracellularis* serum." (Specification, page 28, lines 8-10).

To further that the 75 Kd protein performs as an effective protective antigen when administered in a vaccine in which it is the sole *L. intracellularis* antigen, as stated in the Specification and claimed, an experiment was conducted under my supervision in which the 75 kD protein was the sole component in a vaccine.

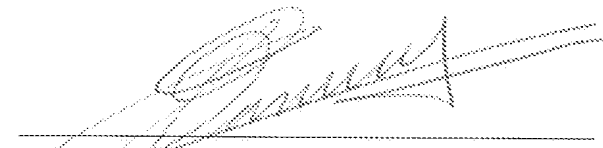
The experiment is described as follows:

6-week-old SPF pigs were used for the experiment. The pigs were allotted to several groups of five pigs each. Group 3 was vaccinated with the 75 KD protein, as currently claimed, and Group 8 was left as an unvaccinated control. The vaccines were formulated in Diluvac Forte adjuvant (commercially available from Intervet/Schering-Plough Animal Health) and were administered IM in the neck at T=0 and at T=4weeks. At T=6weeks all pigs were challenged orally with homogenized infected mucosa. Subsequently all pigs were observed daily for clinical signs of Porcine Proliferative Enteropathy (PPE). At T=0, 6, 7, 8 and 9weeks, all pigs were weighed and serum blood (for serology IFT test) and faeces (for PCR) were sampled. At T=9 weeks all pigs were euthanized and necropsied. The intestines (ileum and jejunum) were checked macroscopically for *Lawsonia intracellularis* infection, as well as microscopically (immunohistology).

The results are in the attached tables. Table 3 illustrates that the vaccine induced a significant anti-body titre (7.3) compared to the control (1.2). Table 5 clearly shows that the vaccine was able to significantly reduce the shedding of *Lawsonia intracellularis* after challenge. Table 7 indicates that the post-mortem scores of the Ileum are also significantly better than those of the control group (24 vs. 100). Table 8 clearly shows that the vaccinated group histologically scored negative after challenge, whereas the control group scored positive.

These results indicate that the presently claimed subject matter solves the problem of obtaining a protective immune response, which is shown can be achieved using only the 75 kD protein.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Paul Vermeij

Date 23-12-2010

Table 3 IFT antibody titre (2log) of pig serum after vaccination en challenge

Group	pig no.	2log IFT titre in 1/4 diluted serum at		
		T=0	T=6w	T=9w
1	1137	4.0	>12.0	>12.0
	1138 ^{day 13}	0.0	4.5	ns
	1202	0.0	10.0	>12.0
	1135	0.0	>12.0	11.5
	1210	0.0	10.5	11.5
	mean	0.8	>9.8	>11.8
2	1280	0.0	0.0	10.5
	1694 ^{day 21}	0.0	0.0	ns
	1227 ^{day 16}	0.0	0.0	ns
	1357 ^{day 14}	0.0	0.0	ns
	1595	0.0	0.0	10.5
	mean	0.0	0.0	10.5
3	1141	0.0	0.0	6.5
	1319	0.0	10.0	9.0
	1356	0.0	9.0	10.0
	1622	4.0	7.5	6.0
	1200	5.0	10.0	9.0
	mean	1.8	7.3	8.1
4	1624	0.0	6.0	8.0
	1139	4.0	5.0	7.5
	1176	3.5	9.5	10.0
	1136	3.0	6.5	8.0
	1322	0.0	8.0	7.5
	mean	2.1	7.0	8.2
5	1641	2.0	0.0	10.0
	1561	0.0	0.0	9.0
	1461	0.0	0.0	8.0
	1168	0.0	0.0	9.0
	1426	0.0	0.0	8.5
	mean	0.4	0.0	8.9
6	1323 ^{day 15}	0.0	0.0	ns
	1267	0.0	0.0	9.0
	1605	0.0	0.0	10.0
	1582	0.0	0.0	7.0
	1318	0.0	6.0	8.0
	mean	0.0	1.2	8.5
7	1474 ^{day 9}	0.0	0.0	ns
	1240	0.0	0.0	>12.0
	1342 ^{day 14}	0.0	0.0	ns
	1308 ^{day 15}	0.0	0.0	ns
	1583	0.0	0.0	11.5
	mean	0.0	0.0	>11.8
8 control	1225	0.0	6.0	8.0
	1612	0.0	0.0	9.5
	1368	0.0	0.0	9.5
	1640	0.0	0.0	>12.0
	1166	0.0	0.0	11.0
	mean	0.0	1.2	>10.0

IFT control sera	pre-dilution	titre
pos pig #147	1/64	8
dd: 08-05-03		
neg pig serum	1/4	0
LIV 9913007		

Table 5 Results PCR on pooled faeces samples

Group	pig no.	PCR on pooled faeces samples (dilution)				
		T=0w	T=6w	T=7w	T=8w	T=9w
1	1137	0	0	100	100	100
	1138 ^{day 13}					
	1202					
	1135					
	1210					
	mean					
2	1280	0	0	10000	10000	10000
	1694 ^{day 21}					
	1227 ^{day 16}					
	1357 ^{day 14}					
	1595					
	mean					
3 75KD	1141	0	100	0	1	0
	1319					
	1356					
	1622					
	1200					
	mean					
4	1624	0	100	0	1	0
	1139					
	1176					
	1136					
	1322					
	mean					
5	1641	0	100	100	100	0
	1561					
	1461					
	1168					
	1426					
	mean					
6	1323 ^{day 15}	0	10000	10000	10000	1
	1267					
	1605					
	1582					
	1318					
	mean					
7	1474 ^{day 9}	0	0	100	10000	100
	1240					
	1342 ^{day 14}					
	1308 ^{day 15}					
	1583					
	mean					
8 control	1225	0	1 ^a	100	10000	100
	1612					
	1368					
	1640					
	1166					
	mean					

^a analysing individual pig samples showed that the positive signal of group 8 was solely due to #1225
1694^{day 21} culled at indicated post-challenge day (end of experiment was at day 24 post-challenge)

Table 7 Post-mortem scores

Group	pig no.	lesion ^a	% ileum affected	total score
1	1137	0	0	0
	1138 ^{day-13}			
	1202	0	0	0
	1135	0	0	0
	1210	2	30	60
	mean			15
2	1280	2	30	60
	1694 ^{day 21}			
	1227 ^{day 16}	2.5	100	250
	1357 ^{day 14}	0	0	0
	1595	3	100	300
	mean			152.5
3 75110	1141	2	30	60
	1319	2	20	40
	1356	2	10	20
	1622	0	0	0
	1200	0	0	0
	mean			24
4	1624	2	10	20
	1139	0	0	0
	1176	2	20	40
	1136	0	0	0
	1322	0	0	0
	mean			12
5	1641	1	50	50
	1561	2	50	100
	1461	1	20	20
	1168	2	100	200
	1426	2	50	100
	mean			94
6	1323 ^{day 15}	2	20	40
	1267	2	30	60
	1605	2	50	100
	1582	2	30	60
	1318	0	0	0
	mean			52
7	1474 ^{day 9}			
	1240	2	100	200
	1342 ^{day 14}	1	50	50
	1308 ^{day 15}	2.5	80	200
	1583	0	0	0
	mean			112.5
8 control	1225	0	0	0
	1612	1	20	20
	1368	2	80	160
	1640	2	80	160
	1166	2	80	160
	mean			100

^a 1=minimal, 2=mild, 3=moderate thickening and/or reddening of ileum mucosa

1694^{day 21} culled at indicated post-challenge day (end of experiment was at day 24 post-challenge)

Table 8 Histology score ileum

Group	pig no.	HE	IHC	total
1	1137	-	-	0
	1138 ^{day 13}	-	-	0
	1202	+	++	3
	1135	(+)	-	0.5
	1210	++	++	4
	mean	3	4	7.5
2	1280	-	-	0
	1694 ^{day 21}	-	-	0
	1227 ^{day 16}	++	++	4
	1357 ^{day 14}	++	++	4
	1595	++(+)	++	4.5
	mean	6.5	6	12.5
3	1141	-	-	0
	1319	-	-	0
	1356	-	-	0
	1622	-	-	0
	1200	-	-	0
	mean	0	0	0
4	1624	-	-	0
	1139	-	-	0
	1176	-	-	0
	1136	-	-	0
	1322	-	-	0
	mean	0	0	0
5	1641	(+)	(+)	1
	1561	-	-	0
	1461	-	-	0
	1168	-	-	0
	1426	-	-	0
	mean	0.5	0.5	1
6	1323 ^{day 15}	-	+	1
	1267	-	-	0
	1605	-	+	1
	1582	-	-	0
	1318	-	-	0
	mean	0	2	2
7	1474 ^{day 9}	ns	ns	ns
	1240	+	++	3
	1342 ^{day 14}	++	++	4
	1308 ^{day 15}	++	++	4
	1583	-	+	1
	mean	5	7	12
8 control	1225	-	-	0
	1612	(+)	++	2.5
	1368	-	-	0
	1640	++	++	4
	1166	-	(+)	0.5
	mean	2.5	4.5	7

(+) = very mild, + = mild, ++ = moderate, +++ = severe

1694^{day 21} culled at indicated post-challenge day (end of experiment was at day 24 post-challenge)